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Phototransduction in Fan Worm Radiolar Eyes

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Title: Phototransduction in Fan Worm Radiolar Eyes

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Keywords: opsin, polychaete, G-protein, GPCR, phototransduction, vision, eyes, phylogenetics

eTOC blurb: Bok et al. explored the phototransduction machinery expressed in the radiolar eyes of the fan worms. Transcriptomics revealed that these unusual eyes express a poorly-understood opsin typically found in invertebrate brains. Adapting this opsin for use in the eyes offers an example of functional flexibility in the evolution of new sensory systems.

Main Text:

Fan worms (Annelida: Sabellidae) are sessile polychaetes that spend their adult lives in tubes and project their fans, composed of radiolar tentacles, up into the water column for respiration and filter feeding. To protect the fan from predation, many species have evolved unique compound eyes on the radioles that function as shadow or motion detectors, eliciting a rapid withdrawal response in reaction to encroaching objects in the water column [1, 2]. The structure of the eyes, their complexity, and their arrangements on the radioles are very diverse among sabellid genera [3] and they display many unusual characteristics, such as ciliary photoreceptors [3, 4] that hyperpolarize in response to illumination [5]. Here we examine the retinal transcriptome of the radiolar eyes from the fan worm, *Megalomma interrupta*. We find that the opsin, the protein component of light sensitive visual pigments, and other phototransduction cascade signaling proteins expressed in these eyes are related to those commonly associated with vertebrate ciliary photoreceptors, as opposed to the rhabdomeric receptors found in the primary eyes of many invertebrates. Alongside the previous anatomical and physiological observations, these results suggest that the radiolar eyes arose independently in fan worms.

Members of this genus *Megalomma* have the largest consolidated compound radiolar eyes amongst the sabellids (Fig. 1A-B) [3]. We used transcriptomic sequencing of *M. interrupta* radiolar eyes in order to identify expressed phototransduction cascade components (Fig. 1A). Sequencing yielded a single opsin transcript from *M. interrupta* (Table S1). We created a maximum likelihood phylogeny of metazoan opsins including the new transcript from *M. interrupta* (Figs 1C, S1A).

Opsins phylogenies typically recover four major opsin clades, c-opsins, tetraopsins, xenopsins, and Gq-opsins [reviewed in 6, 7]. The *M. interrupta* opsin sequence falls out within the c-opsin clade in a cluster of poorly-understood basally-branching invertebrate sub-clades which we refer to as invertebrate c-opsins (InvC). These include c-opsins from panarthropods, echinoderms, and the marine ragworm *Platynereis dumerilii*, with which the *M. interrupta* radiolar eye opsin is most homologous (Figs. 1E, S1C). The c-opsin clade is otherwise dominated by chordate visual, neural, and extraocular opsins. The *P. dumerilii* InvC-opsin is expressed in simple ciliary photoreceptors in the brain of larvae for an unclear function likely restricted to luminance assessment [8]. Other InvC-opsins have been localized to neuronal tissue in the brains or distributed sensory networks of honey bees, velvet worms, spiders, horseshoe crabs, and echinoderms (Figs. 1E, S1C). This unusual example of a InvC-opsin being the main photopigment in an eye is likely a unique elaboration of fan worms. The origins and typical function of neuronally-expressed InvC-opsins remain elusive.

We also identified g-protein α-subunit transcripts from the radiolar eyes (Fig. 1D). G-proteins convey the cellular signal from the opsin to the downstream phototransduction cascade, and certain types of g-protein α-subunits often interact with specific opsins [6]. For instance, c-opsins typically signal using a $G\alpha_i$, such as transducin in vertebrate rods and cones, and the Gq-opsins in rhabdomeric photoreceptors, such as those in the primary eyes of *P. dumerilii*, have invariably been observed to signal through a $G\alpha_q$ (Fig S1A, 1C-D). We found that the dominant g-protein α-subunit transcript in *M. interrupta* radiolar eyes is a $G\alpha_o$ (Fig 1D, Table S1). Though we cannot definitively confirm interaction from transcriptomic sequencing alone, this $G\alpha_o$ is the first g-protein to be implicated in signaling with an InvC-opsin. $G\alpha_o$ was previously only observed in the distributed visual system of scallops in conjunction with a tetraopsin expressed in the ciliary retina [9], and in the lizard parietal eye in conjunction with a chordate non-visual c-opsin [10]. Considering these examples of opsin-$G\alpha_o$ interaction in diverse phyla, it is fascinating to consider their evolutionary or functional associations.

A number of transcripts coding for further downstream ciliary phototransduction cascade components were also recovered, including G-protein β and γ subunits (Table S1). We note that

sequencing also identified a $G\alpha_i$ transcript from *M. interrupta*, but it was weakly expressed and is likely not responsible for phototransduction in the retina (Table S1).

What factors may have resulted in the presumptive application of this unusual InvC-$G\alpha_o$ phototransduction cascade to the radiolar eyes of fan worms? The answer may be a combination of functionality and pragmatism. Hyperpolarizing ciliary photoreceptors make excellent shadow detectors for a sessile animal, with a decrease in light eliciting a proper depolarizing sensory response [5]. Thus, for the simple task of detecting decreases in light intensity, a c-opsin cascade could be advantageous compared to a Gq-opsin cascade that depolarizes in response to increasing light. Therefore, evolution made use of what was available, and a non-visual, neuronal InvC-opsin was apparently recruited for the radiolar eyes. We define non-visual opsins as opsins not currently known to be involved in directional, motion-detecting, or spatially-resolving visual tasks. Given the number of different non-visual opsin homologues available to most bilaterian phyla [7], it is perhaps not surprising that a need for photoreceptors on evolving radioles was most naturally served by a previously non-visual opsin. It is probable that the radiolar eyes then arose independently of other known visual systems, as the InvC-opsin-expressing photoreceptors in fan worm radioles were sequestered within pigment cups providing directionality to their light response. Such simple scattered ocelli are found in many species of fan worms [3], and in some genera, such as *Megalomma*, the individual ocelli are consolidated into true compound eyes providing the potential for further visual capabilities such as motion detection or perhaps even resolving vision.

Many questions still remain regarding the evolution and function of the radiolar eyes of fan worms. However, the diverse, independent elaborations of these eyes in service to an apparently-singular visually guided task make them fascinating targets to explore. Further studies into their development and neuroanatomy can provide unique insights into the evolution of complexity in visual systems, the recruitment of previously non-visual opsins or photoreceptors to new visual tasks, and the emergence of new sensory modalities and visually guided behaviors. Furthermore, we must attempt to better understand the original functions of the different opsin clades in ancestral bilaterians in order to resolve the evolutionary pathways leading to their present roles.

Supplemental Information

Supplemental Information includes experimental procedures, one figure, and one table and can be found with this article online at *bxs.

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Author Contributions: MJB designed the project, collected and prepared the tissue, assisted with data analysis, and wrote the manuscript. MLP extracted RNA for transcriptomics, analyzed the phylogenetic data and assisted with editing the manuscript. DEN oversaw the project and assisted with editing the manuscript.

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Figure Legends:

Figure 1: Phototransduction components expressed in a fan worm radiolar eye.

(A) *Megalomma interrupta* emerging from its tube, prominently displaying the two compound eyes on the dorsal-most radioles (arrowhead).

(B) Focal-stack micrograph of a radiolar eye. Scale bar: 100 μm.

(C) A metazoan opsin phylogeny cladogram indicating the four major opsin clades (after [6,7]) and the transcripts found in the radiolar eyes of *M. interrupta* (blue circle) and primary eyes of *Platynereis dumerilii* (green circle).

(D) A phylogeny of G-protein α-subunits implicated in animal visual systems. *M. interrupta* radiolar eyes primarily expresses a $G\alpha_o$. The Gα clades are colored according to the opsin clade (C) that they are commonly associated with.

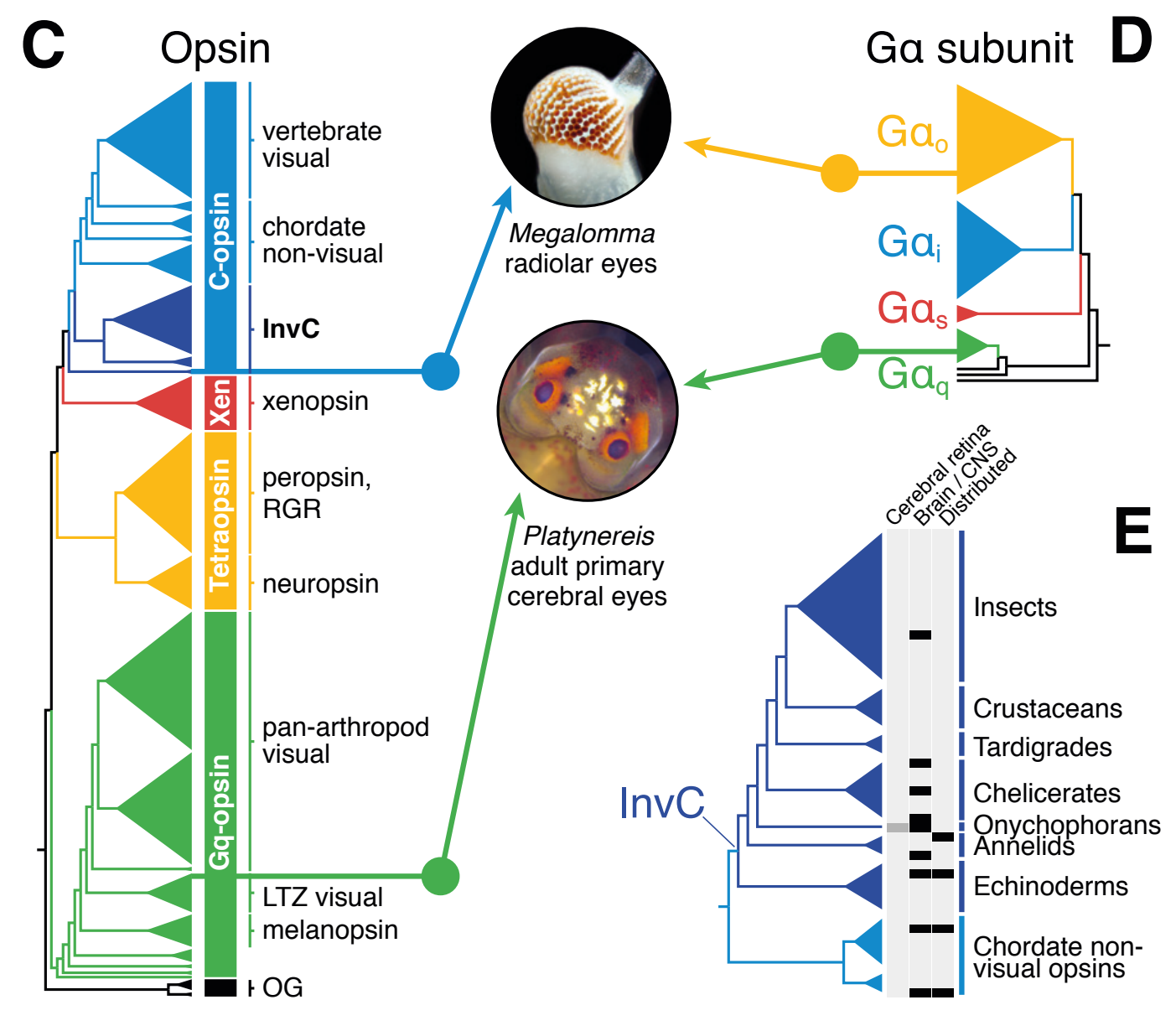
(E) An expanded phylogeny of the Invertebrate C opsin (InvC) clade indicating included taxa and known tissue expression. Black squares, expressed; grey, ambiguous; light grey, unknown. See Figure S1 for full phylogenetic trees, and expression location references.

Supplemental Information

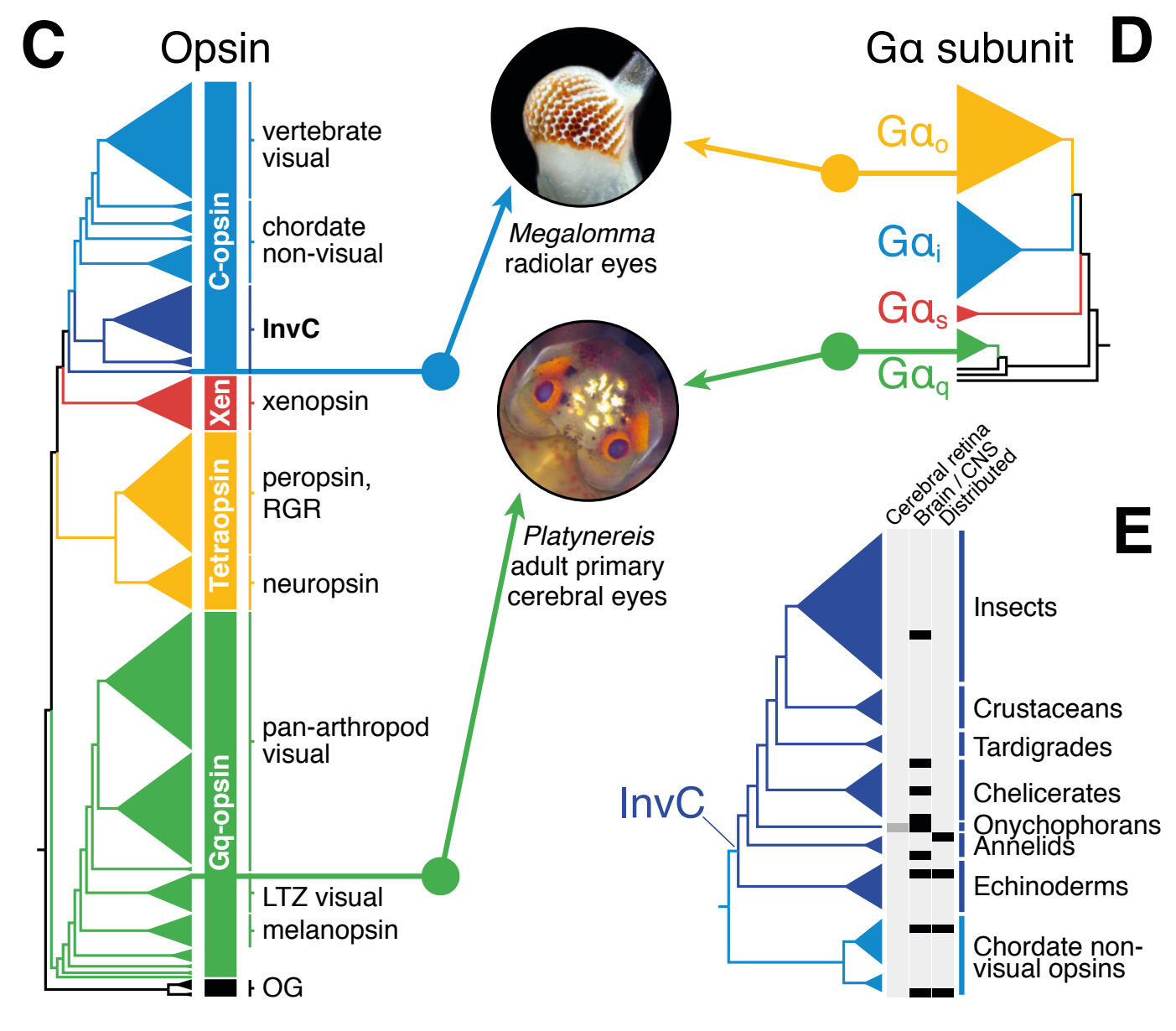
Document S1. Experimental Procedures and One Table.

Figure S1. Related to Figure 1. Phylogenetic trees of metazoan opsins **(A)**, g-protein α subunits **(B)**, and invertebrate C-opsins **(C)**. Annelid sequences are indicated by thick branches, and circles indicate sequences that are highlighted in Figure 1C-E. All legends refer to substitutions per site (over the branch length indicated). Panel A contains labels for g-protein interaction information adapted from [8]. Panel C includes citations to supplemental references in brackets regarding tissue expression sites of the various InvC-opsins.


A
B
fan
tube
head
(inside tube)


C
Opsin
D
Gα subunit
vertebrate
visual
chordate
non-visual
InvC
C-opsin
xenopsin
Xen
peropsin,
RGR
neuropsin
Tetraopsin
pan-arthropod
visual
Gq-opsin
LTZ visual
melanopsin
OG
Megalomma
radiolar eyes
Platynereis
adult primary
cerebral eyes
Gαo
Gαi
Gαs
Gαq
E
Cerebral retina
Brain / CNS
Distributed
Insects
Crustaceans
Tardigrades
Chelicerates
Onychophorans
Annelids
Echinoderms
Chordate non-
visual opsins
InvC


A
B
fan
tube
head
(inside tube)


C
Opsin
vertebrate visual
chordate non-visual
InvC
C-opsin
Xen
xenopsin
Tetraopsin
peropsin, RGR
neuropsin
Gq-opsin
pan-arthropod visual
LTZ visual
melanopsin
OG
Megalomma radiolar eyes
Platynereis adult primary cerebral eyes
D
Gα subunit
Gαo
Gαi
Gαs
Gαq
E
Cerebral retina
Brain / CNS
Distributed
Insects
Crustaceans
Tardigrades
Chelicerates
Onychophorans
Annelids
Echinoderms
Chordate non-visual opsins
InvC

Supplemental Information: Phototransduction in Fan Worm Radiolar Eyes

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Supplemental Experimental Procedures

Animals: *Megalomma interrupta* were collected at Lizard Island Research Station in Queensland, Australia (-14.694150, 145.461987) at 1-2 meters depth from dead, encrusted branching coral rubble in accordance with the University of Queensland Limited Impact Accreditation Collecting Permit No. UQ001/2014. They were identified anatomically (according to [S9]).

Photography: Four Figure 1B, dorsal radiolar eyes were dissected and imaged using a 5X objective at 20 to 30 focal planes. The images were focus stacked using Zerene Stacker software (Zerene Systems LLC, Richland, WA, USA), and the resulting image was adjusted for contrast, brightness and white balance using Adobe Photoshop Lightroom 5 (Adobe Systems Incorporated, San Jose, CA, USA).

Tissue: The radiolar eyes were dissected from 6 individuals in the field by cutting the radioles directly below the base of the eye. The 12 eyes were pooled and stored in RNA Later for transport to the lab where the total RNA was extracted using the Nucleospin RNA XS kit (Machery-Nagel).

RNAseq: The total RNA was quantified using a Qubit Fluorometer and sent to Molecular Research LP (Shallowater, TX) for library construction and sequencing of 300bp paired end reads on a Miseq (Illumina), resulting in 7.4 million reads. Raw reads were quality checked and de novo assembled using the DNAStar software package. The resulting assembly included 5.0 million reads (67.6%), consisting of 6075 contigs with an average coverage of 13X and an N50 of 1036 bp. Phototransduction genes in the assembly were identified using phylogentically informed annotation [S10]. Expression levels were calculated as FPKM using read mapping in BowTie2 v2.2.3, run on the NCGAS Mason Linux Server (Indiana University, Bloomington, IN USA).

Phylogeny reconstruction: Opsin and G-protein alpha subunit trees were reconstructed by aligning *M. interrupta* sequences with a selection from the known diversity of groups within each gene family. Amino acid sequences were aligned using MAFFT [S11] and trees were reconstructed using RAxML v8 [S12] as implemented on the CIPRES portal [S13].

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Supplemental Tables

Table S1. Related to Figure 1. Phototransduction cascade transcripts identified by using phylogenetically informed annotation in the radiolar eye retinal transcriptome of *Megalomma interrupta*.

Gene	*Megalomma* Contig	Length (AA)	Expression level (FPKM)	Acc. #
C-opsin	Contig719	354	217.8	MF145115
G(o) alpha subunit	Contig1351	375	72.3	MF145114
G(i) alpha subunit	Contig5021	310	0.0	MF145116
G-protein beta subunit	Contig15	351	2395.9	MF145125
G-protein gamma subunit	Contig5252	86	0.0	MF145117
	Contig347	90	293.5	MF145121
	Contig293	90	667.9	MF145122
	Contig248	90	325.9	MF145123
Cnga1	Contig5874	482	0.0	MF145118
RGS9	Contig2543	165	39.2	MF145126
rdgA / DAGK (PKC)	Contig5349	288	25.5	MF145127
	Contig4414	231	32.5	MF145128
Rcvrn (recoverin)	Contig127	162	1034.5	MF145113
	Contig174	100	337.6	MF145129
	Contig210	100	292.0	MF145130
	Contig2326	161	23.0	MF145119
	Contig3575	178	0.0	MF145120
	Contig3596	191	35.2	MF145131
	Contig5812	170	23.4	MF145124

Supplemental Figure Legends

Figure S1. Related to Figure 1. Phylogenetic trees of metazoan opsins **(A)**, g-protein α subunits **(B)**, and invertebrate C-opsins **(C)**. Annelid sequences are indicated by thick branches, and circles indicate sequences that are highlighted in Figure 1C-E. All legends refer to substitutions per site (over the branch length indicated). Panel A contains labels for g-protein interaction information adapted from [8]. Panel C includes citations to supplemental references in brackets regarding tissue expression sites of the various InvC-opsins.

Figure S1

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Supplemental Movies and Spreadsheets

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